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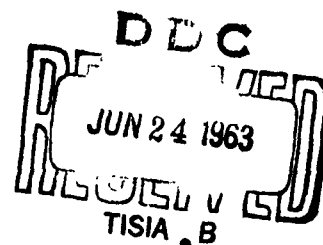
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CHANGES IN THE INTESTINAL FLORA OF GROUND  
SQUIRRELS DURING PERIODS OF HIBERNATION

J. P. Schmidt  
R. E. Becker

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## ABSTRACT

Counts were made of the coliform bacilli and psychrophilic organisms in the intestinal contents of arctic ground squirrels while the animals were in the active state and again following periods of hibernation. In addition, total viable aerobic cell counts were obtained. Data are presented which indicate that there was a significant increase in the number of psychrophiles and a simultaneous decrease in the number of coliform bacilli during hibernation. No changes were found in the total cell counts which could be associated with hibernation.

## PUBLICATION REVIEW

  
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HORACE F. DRURY  
Director of Research

# CHANGES IN THE INTESTINAL FLORA OF GROUND SQUIRRELS DURING PERIODS OF HIBERNATION

## SECTION 1. INTRODUCTION

During hibernation the body temperature of ground squirrels drops from a normal of approximately  $36^{\circ}\text{C}$  to a point near  $0^{\circ}\text{C}$ . Within a limited range their body temperature parallels that of the environment, usually remaining  $0.5^{\circ}$  to  $3.0^{\circ}\text{C}$  above the environmental temperature (Johnson, 1931). Mayer (1958) found that the average rectal temperature of ground squirrels hibernating in an environmental temperature of  $1.7^{\circ}\text{C}$  was  $4.2^{\circ}\text{C}$ . Under these conditions it might be expected that those microorganisms which are capable of reproduction at such reduced temperatures, if present in the animal, would increase proportionately over those requiring a higher incubation temperature. Accordingly, studies were undertaken during the winter of 1959-1960 to determine the number of psychrophiles and coliform bacilli in the intestinal contents of arctic ground squirrels and to record any quantitative changes which might be associated with hibernation.

## SECTION 2. MATERIALS AND METHODS

The arctic ground squirrels (*Spermophilus undulatus*) were captured in the Paxson Lake area of central Alaska during the month of August. They were housed individually in metal cages without nesting material and fed a diet of fresh lettuce, carrots and Friskies dog cubes. Fifteen adult animals were utilized and they were observed in the Laboratory animal colony for two months before the studies were initiated. Hibernation was induced by lowering the ambient temperature to  $3^{\circ} \pm 1^{\circ}\text{C}$  and that temperature was maintained throughout the study period. To determine the hibernating experience of the animals a small amount of sawdust was sprinkled on their backs after they had become completely dormant. So long as the sawdust remained in place it was assumed that the animal had been in continuous hibernation. The animals were observed daily but not disturbed unnecessarily because various stimuli will often trigger an arousal from the hibernating state.

Fresh fecal droppings were collected on clean filter paper sheets positioned under the wire mesh floor of the cages. Droppings contaminated with voided urine were not included. The specimens were kept at 4° C until processing, which took place within two hours after defecation. The droppings from an individual animal were weighed and combined with an equal part (weight) of sterile distilled water. From this a uniform suspension was made with the aid of a sterile glass rod. Part of the material was used for the determination of the per cent moisture and another portion for the preparation of cultures.

Triplicate cultures were inoculated from serial 10-fold dilutions ( $10^{-3}$  to  $10^{-8}$ ) of the specimens and the results obtained were calculated as the number of organisms per gram dry weight of fecal material.

The coliform bacilli were cultured on (Difco) eosin methylene blue agar at 37° C. The plates were seeded with 0.1 ml of the appropriate dilution which was then spread over the surface of the agar with a bent glass rod. The inoculum was accurately measured and dispensed with a sterile 0.1 ml pipette. Using this technique well-isolated colonies were consistently obtained. All gram negative rods yielding lactose-positive colonies within 48 hours were arbitrarily considered to be coliform bacilli.

For the enumeration of the psychrophiles (Difco) tryptone glucose extract (TGE) agar was employed. Pour plates were prepared using an inoculum of 1.0 ml and the incubation was carried at 2° to 3° C for 14 days. Organisms producing a visible colony within 14 days were termed psychrophiles. In addition, pour plates were made in TGE agar as above except that these cultures were incubated at 37° C for 48 hours. The results thus obtained were referred to as the "total count" at 37° C. All counts were made using a Quebec colony counter and only those plates having between 30 and 300 colonies were included.

### SECTION 3. RESULTS AND DISCUSSION

The data (Figure 1) indicated a significant increase in the number of psychrophiles and a simultaneous decrease in the number of coliform bacilli following periods of hibernation. On the other hand, very little fluctuation was observed in the total counts at 37° C, and these counts did not appear to be influenced in any way by hibernation. The values shown in Figure 1 represent the averages obtained with the 15 ground squirrels

studied. Although there was some variation in the hibernation habits, activity, food consumption and normal intestinal flora of the individual animals, each of the squirrels studied demonstrated these characteristic trends.

A representative number of the coliform bacilli and psychrophiles were isolated and their temperature-growth relations determined. None of the coliform bacilli tested grew at temperatures below 5° C. This would imply that little or no multiplication of these organisms occurred in the intestinal tract of the animals while they were in the hibernating state and it would be consistent with the decrease in the coliform count shown in Figure 1. The psychrophiles grew well when incubated at temperatures down to 0° C. Although Ingrahm and Stokes (1959) reported that some psychrophilic bacteria have a maximum growth temperature above 37° C, none of the psychrophiles isolated in our study gave evidence of growth at temperatures above 35° C.

Ground squirrels and other hibernating mammals undergo natural awakenings from time to time during the hibernating period (Lyman and Chatfield, 1955). Laboratory observations have shown that the duration of continuous hibernation varies from species to species. In this study the average was 6.0 days with extremes of 2 and 19 days. Between periods of hibernation the animals were active for an average of 1.3 days, indicating that they were in hibernation approximately 82 per cent of the time. This figure must be considered conservative because it takes an active animal from eight hours to several days to become completely dormant and the waking process requires about three hours (Lyman and Chatfield, 1955).

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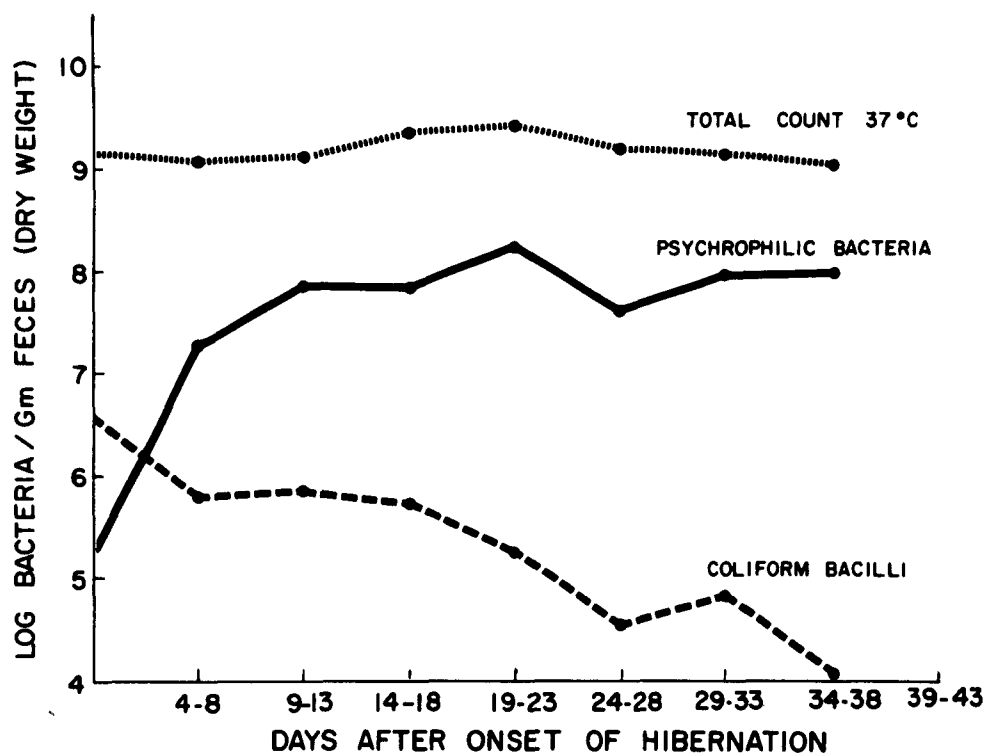


FIGURE 1

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2. Johnson, G. E. Hibernation in mammals. Quart. Rev. Biol. 6:439-461, 1931.
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4. Mayer, W. V. Studies on the arctic ground squirrel. Final Report, AF 18(600)-843, Arctic Aeromedical Laboratory, APO 731, Seattle, Washington, 1958.



<p>Arctic Aeromedical Laboratory, United States Air Force (AFSC), APO 731, Seattle, Wash. Rpt. AAL-TDR-62-55. CHANGES IN THE INTESTINAL FLORA OF GROUND SQUIRRELS DURING PERIODS OF HIBERNATION. March 1963, 5 p. incl. illus., 4 refs. Unclassified Report</p> <p>Counts were made of the coliform bacilli and psychrophilic organisms in the intestinal contents of arctic ground squirrels while the animals were in the active state and again following periods of hibernation. In addition, total viable aerobic cell counts were obtained. Data are presented which indicate that there was a significant increase in the number of psychrophiles and a simultaneous decrease in the number of coliform bacilli during hibernation. No changes were found in the total cell counts which could be associated with hibernation.</p>	<ol style="list-style-type: none"> <li>I. Intestines</li> <li>2. Bacteria</li> <li>3. Rodents</li> <li>4. Hibernation</li> <li>I. Project 8241-12</li> <li>II. Schmidt, J. P. and R. E. Becker</li> <li>III. Available from OTS</li> <li>IV. In ASTIA collection</li> </ol>
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